

## ULTRA-VIOLET LIGHT EMISSION FROM HPV-G CELLS IRRADIATED WITH LOW LET RADIATION FROM $^{90}\text{Y}$ ; CONSEQUENCES FOR RADIATION INDUCED BYSTANDER EFFECTS

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□ In this study, we aimed to establish the emission of UV photons when HPV-G cells and associated materials (such as the cell substrate and cell growth media) are exposed to low LET radiation. The potential role of UV photons in the secondary triggering of biological processes led us to hypothesize that the emission and absorption of photons at this wavelength explain some radiation induced “bystander effects” that have previously been thought to be chemically mediated. Cells were plated in Petri-dishes of two different sizes, having different thicknesses of polystyrene (PS) substrate, and were exposed to  $\beta$ -radiation from  $^{90}\text{Y}$  produced by the McMaster Nuclear Reactor. UV measurements were performed using a single photon counting system employing an interference-type filter for selection of a narrow wavelength range,  $340 \pm 5$  nm, of photons. Exposure of the cell substrates (which were made of polystyrene) determined that UV photons were being emitted as a consequence of  $\beta$  particle irradiation of the Petri-dishes. For a tightly collimated  $\beta$ -particle beam exposure, we observed 167 photons in the detector per unit  $\mu\text{Ci}$  in the shielded source for a 1.76 mm thick substrate and 158 photons/ $\mu\text{Ci}$  for a 0.878 mm thick substrate. A unit  $\mu\text{Ci}$  source activity was equivalent to an exposure to the substrate of 18  $\beta$ -particles/ $\text{cm}^2$  in this case. The presence of cells and medium in a Petri-dish was found to significantly increase (up to a maximum of 250%) the measured number of photons in a narrow band of wavelengths of  $340 \pm 5$  nm (i.e. UVA) as compared to the signal from an empty control Petri-dish. When coloured growth medium was added to the cells, it reduced the measured count rate, while the addition of transparent medium in equal volume increased the count rate, compared to cells alone. We attribute this to the fact that emission, scattering and absorption of light by cells and media are all variables in the experiment. Under collimated irradiation conditions, it was observed that increasing cell density in medium of fixed volume resulted in a decrease in the observed light output. This followed a roughly exponential decline. We suggest that this may be due to increased scattering at the cell boundary and absorption of the UV in the cells. We conclude that we have measured UVA emitted by cells, cell medium and cell substrates as a consequence of their irradiation by low LET  $\beta$ -particle radiation. We suggest that these secondary UV photons could lead to effects in non-targetted cells. Some effects that had previously been

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attributed to a chemically mediated “bystander effect” may in fact be due to secondary UV emission. Some radiation bystander effect studies may require re-interpretation as this phenomenon of UV emission is further investigated.

*Keywords:- IBIL, HPV-G cells, Ultraviolet, Luminescence,  $\beta$ -particles*

## INTRODUCTION

Deviation from the linear-no-threshold (LNT) theory for very low doses of radiation has been a subject of debate amongst the scientific community for some time. Various effects have been observed that include increased sensitivity of cells, both towards protection against further radiation insult, and toward more cell death (Matsubara *et al.* 2000, Sawant *et al.* 2001, Schettino *et al.* 2001). Amongst these low radiation dose study observations, the effects on the non-targeted cells have been studied quite extensively, and are usually described as “radiation induced bystander effects” (Mothersill and Seymour 2001). This phenomenon not only challenges the LNT model for low doses of radiation, but also indicates the fact that damage to the cell nucleus is not a basic criterion for producing overall damage to the cell (Lorimore *et al.* 2003). Cellular irradiation in bystander effect studies is often performed using micro-beams that can deliver a quantified (and small) number of particles to specified cells. An alternative method to microbeams used in bystander effect studies is where a very low fluence of charged particles is employed, so that only a small proportion of the overall population of cells is traversed by primary charged particle tracks. Irrespective of the method of irradiation cells are plated on a variety of different substrate materials such as polypropylene, mylar or polystyrene that could fluoresce upon irradiation.

It is a well-known fact that charged particles, while passing through media create dense ionizations and excitations. In organic materials the neutralization or de-excitation of the ionized/excited molecules can occur in a variety of different ways that include subsequent emission of radiation at different frequencies, from x-ray through to UV and optical frequencies and also production of heat in the medium. Electrons are also known to produce bremsstrahlung radiation when their trajectory is altered around a nuclear field (or they are decelerated in the medium). In addition, in the case of electrons passing through a dielectric medium, with velocities greater than the phase velocity of light in that medium, Cerenkov light can be observed as a result of de-excitation of the molecules. If the refractive index of tissue is considered to be 1.4 then the minimum energy required of  $\beta$  particles to generate Cerenkov light is 0.219 MeV (Axelsson *et al.* 2011). In this experiment, we used  $\beta$ -particles from Y-90 where the end point energy is 2.28 MeV and the average  $\beta$  energy was 0.93 MeV. This energy of  $\beta$ -particles would certainly create Cerenkov photons. However the energy lost through the Cerenkov process is much

less than that lost by ionizations (40 keV/m in case of Cerenkov energy loss and 200 MeV/m in case of ionization energy loss (Rohlf 1994)). The purpose of our experiment was to measure the level of UV photons detectable outside of irradiated cells and medium. The mechanism of generation of the UV (whether it be from ionizations or through the Cerenkov interaction) is not really relevant to our purpose. We wished to determine the production of photons at particular wavelengths which could subsequently lead to the production of a biological response upon absorption by cells.

In simple polymeric structures, or other simple molecular structures, it is possible that the emission and absorption bands do not overlap if the de-excitation occurs in the form of electromagnetic radiation. However, when the molecular structures become more and more complex, such as in the case of cells and their media, there is a strong chance that emission and absorption bands overlap for a wide range of electromagnetic frequencies. This means, for example, that if UV emission occurs in the cell culture as a consequence of irradiation, then the UV photon can be re-absorbed elsewhere in the cell culture and perhaps lead to effects at a distance from the original site of irradiation. It becomes important not to ignore the potential consequences of re-absorption of this UV radiation in the surrounding medium in the conceptualization of “bystander” experiments.

In previous studies, we have demonstrated considerable production of light (including UV frequencies) from a variety of experimentally relevant cellular substrate materials as a consequence of charged particle irradiation (Ahmad *et al.* 2012). Emission frequencies were found to lie within the visible and ultra-violet ranges. Several other studies have also shown the production of light in the UV and visible ranges when experimentally relevant materials (and polymers) are irradiated with charged particles (Pallon *et al.* 1997, Rossi *et al.* 2001, Quaranta *et al.* 2002). However, we wished specifically to study whether cells and cell media would also emit light, especially in the UV range, as a consequence of irradiation. This fluorescent emission of light at UV frequencies can occur both for high and low LET radiation. This is, of course, the basis of liquid scintillation counting: light is emitted in certain organic materials (liquid scintillators) when either energetic electrons or other charged particles are mixed with them. (Krajcar *et al.* 2009, Bhade *et al.* 2010). Our previous studies made use of proton beams (high LET radiation) to irradiate experimentally relevant materials. The irradiation was performed under vacuum which is not feasible for cells and cell media. We therefore performed this study to quantify the UV emission as a consequence of irradiation of cells and cell media by low LET  $\beta$ -particles.

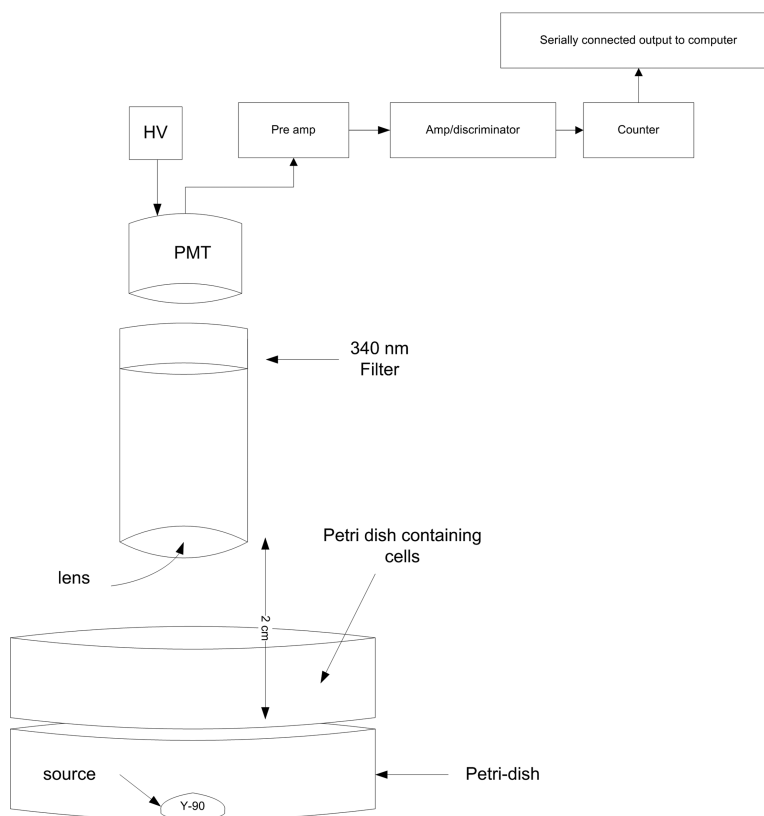
## MATERIALS AND METHODS

### HPV-G cell culture

We used the HPV-G cell line due to its extensive use in radiation induced bystander effect studies (James *et al.* 2005, Lyng *et al.* 2006, Ryan *et al.* 2008). HPV-G cells are immortalised human skin keratinocytes, originally obtained from Dr J. DiPaolo at National Institute of Health. Cell cultures were performed in a bio-safety cabinet level II. We used a cell growth medium (RPMI 1640 by Gibco) having a composition of DMEM/F12 medium containing 60 ml FBS, 5 ml penicillin-streptomycin, 5 ml L-glutamine, 15 mM Hepes buffer, and 1 mg/ml hydrocortisone. Cell cultures were kept in T75 flasks until they were 90-95% confluent. Using 0.25% w/v trypsin/1 mM EDTA solution (1:1) the cells were removed from the flasks and were placed in an incubator for 8 to 10 minutes for a complete detachment. In order to neutralize trypsin, 10ml of growth medium was used. The detached cells were re-suspended in medium, and an aliquot was counted using a Z2 Coulter Particle Count and Size Analyzer. Appropriate numbers of cells were then plated in either 35 × 10 mm or 100 × 15 mm sterilized dishes. Cells were then harvested with either 3 ml or 10 ml RPMI 1640 medium for small and large dishes respectively. After 6 hours of incubation at 37° C, the cells were checked, under a microscope, to see if they were attached to the dishes. The medium from the attached cells was then removed carefully. Due to McMaster University's biosafety requirements cells were killed by adding a 70% ethanol solution to them. After 5-10 minutes, the ethanol solution was carefully removed, and the cells were transported to the physics laboratory for irradiation and light counting.

### Source preparation (<sup>90</sup>Y)

We used <sup>90</sup>Y as a source of energetic electrons for our experiments. <sup>90</sup>Y is considered to be an almost pure beta particle emitter with a 100% beta yield with an average energy of 0.9337 MeV. The end point energy of the emitted  $\beta$ -particles is 2.28 MeV. This emission is accompanied by two other  $\beta$ -particle energies and 3 different gamma rays; however their yield is so very low as to usually be considered negligible. The <sup>90</sup>Y source was prepared using standard irradiation procedures at the McMaster Nuclear Reactor (MNR). A flame-sealed quartz tube containing yttrium salt was irradiated in the reactor core then transferred to a fume hood in our controlled High Level laboratories. The activity present in the sample was quantified in a dose calibrator. After scoring and snapping open the quartz tube, behind Plexiglas shielding in a fume hood, the yttrium salt was dissolved in appropriate media. The resulting solution was dispensed into a glass vial which was placed in a shielded container. Due to the highly energetic nature of the  $\beta$ -particles emitted from <sup>90</sup>Y, the source han-



**FIGURE 1.** Schematic diagram of the setup for cell irradiation using  $^{90}\text{Y}$  source in the liquid form and measurement of light output using single photon counting instrumentation

dling was performed in the fume hood behind a 1.3 cm thick Lucite shield.

### **Irradiation and photon counting**

Figure 1 shows the schematic diagram for the general irradiation configuration. The whole set-up shown in Figure 1 was kept inside a light tight aluminum chamber which was placed in a fume hood. The source ( $^{90}\text{Y}$ ) was received in a liquid form. It was placed in a 35 mm diameter (or in some cases a 100 mm diameter) Petri-dish. There was a 10-15 mm air gap between the source and the bottom of the Petri-dish containing the cells. Cells were attached to the bottom of one of two types of polystyrene Petri-dishes which had a substrate thickness of either  $0.878 \pm 0.006$  mm or  $1.760 \pm 0.005$  mm.

We used a single photon counting system for the detection of UV light. This system employs a 16 mm diameter single photon counting photomultiplier tube (R7400P) by Hamamatsu. The PMT was operated at -800 V using a standard NIM based power supply from ORTEC. The out-

put of the PMT was processed by an ORTEC model 9301 preamplifier, whose output was fed to an ORTEC model 9302 amplifier/discriminator and subsequently to the counter (ORTEC model 994). The maximum count rate that can be handled for negative input pulses in the counter was  $100 \times 10^6$  counts/s and  $25 \times 10^6$  counts/s for positive input pulses. The counter can be hooked up to a computer through a serial port. We performed all the experiments in conditions where the PMT dark current was very low i.e. of the order of 4-10 cps. Each data point shown in the results represents an average of 5 to 10 different measurements.

The photons were collected using a band pass interference-type filter from Edmond optics. The filter has a FWHM of 10 nm around a centre wavelength of 340 nm and thus should only permitting photons in the UVA range to pass through the filter. The lens requires a collimated beam of photons in order to obtain the manufacturer claimed efficiency of 25%. For this purpose we used a UV-Vis fused silica lens having a focal plane at a distance of  $1.8 \pm 0.2$  cm. The cellular material was kept approximately 2 cm away from the lens in order to collimate the photons. Since the beta particles generated by the source are energetic enough to reach the lens, this configuration results in a measurement where the total luminescence observed by the PMT comes in part from the lens of the collimating system.

The irradiation of the HPV-G cell samples was performed in two different orientations.

#### ***“Poor” geometry condition***

The general irradiation configuration shown in Figure 1 can be considered as a “poor” geometry condition because the whole Petri-dish is being irradiated, and a significant number of measured photons are scattered photons that did not originate from the focal point of the lens, but do reach the lens.

#### ***“Good” geometry condition***

In order to reduce the significant contribution of scattered photons reaching the lens, we used an aluminum collimator which helped to establish a relationship between the cell density and light output for a fixed irradiance. The aperture (2 mm diameter) in the collimation shield allowed the electron interaction at a small fixed spot in the cell-containing dishes, but reduced the contribution of scattered photons in the collection system.

In both the poor and good geometry conditions we determined the electron flux reaching the Petri-dish containing the cells and the lens of the collimating system using the Geant4 Monte-Carlo toolkit. The source was considered to have a semi-ellipsoidal shape and a Gaussian energy distribution with a mean of 1.12 MeV and a Gaussian width,  $\sigma$ , of 0.4 MeV.

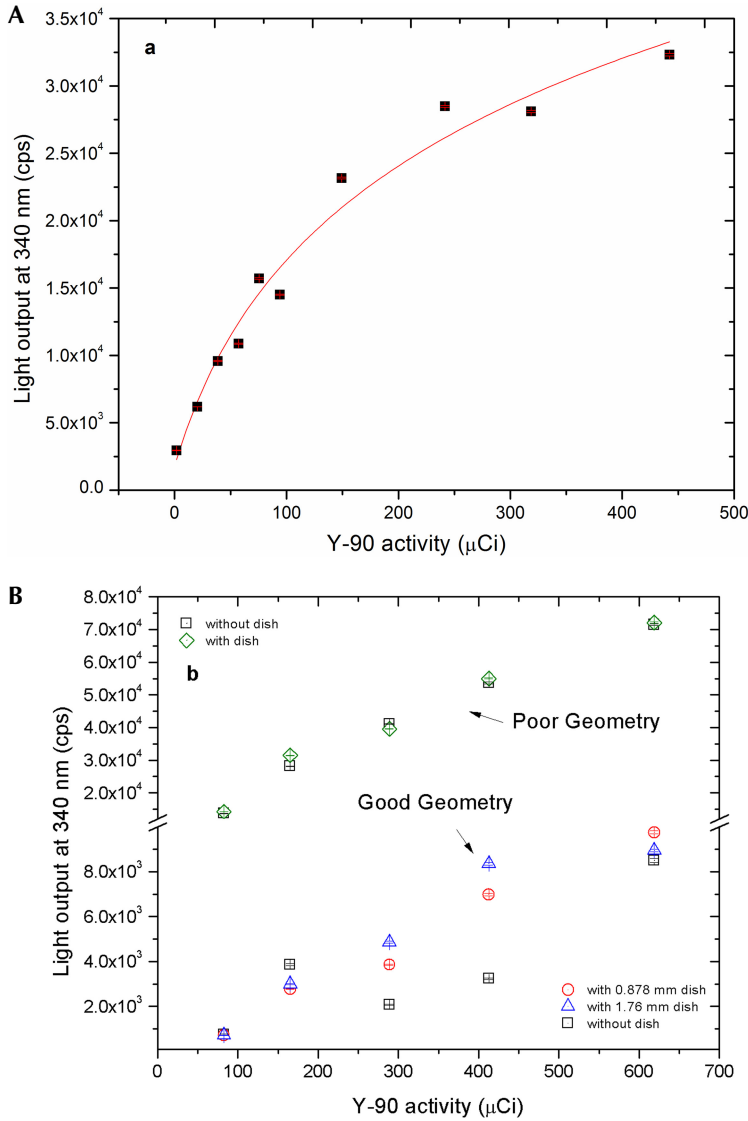
This modeling of the source energy as a Gaussian is an approximation: the  $\beta$  particles will be emitted from the  $^{90}\text{Y}$  in a Fermi distribution and this energy distribution will be modified because the  $^{90}\text{Y}$  is dissolved in solution. We suggest, however, that the use of a Gaussian model around the mean source energy as a first approximation of the  $\beta$  particle energy distribution at the surface of the solution permits a preliminary estimate of the flux reaching the lens.

### **Sources of error**

One of the sources of random error in our experiments was the use of differently prepared specific activities for  $^{90}\text{Y}$  in different experiments. This error would be introduced due to the fact that for the same nominal activity the source volume may be different in different experiments and this could change the irradiation characteristics of the samples. The volume varied from 200  $\mu\text{l}$  to 1 ml for activities ranging from 500  $\mu\text{Ci}$  to 1 mCi. A uniform irradiation would be expected when the source is intact with high specific activity in the form of a point source. In addition we also had a systematic error due to the limited transmission of photons from the lens and filter. The lens transmits 90% of the light over a wide range of electromagnetic spectrum; however the filter has a transmission efficiency of 25% when it is illuminated with a collimated beam of light.

### **RESULTS**

We irradiated blank dishes, that is, Petri-dishes without any cells and cell medium, for a range of  $^{90}\text{Y}$  activities in both the good and poor geometry conditions. The luminescence observed was used to set a control or base-line luminescence. The end point energy of  $^{90}\text{Y}$   $\beta$ -particles allowed some particles to travel up to 1.2 cm (using the Continuous Slowing Down Approximation, CDSA, range) in polystyrene (PS) before losing all of their energy. This meant that after depositing some energy in the polystyrene cellular substrate (which was 0.878-1.76 mm thick), some particles will pass through and interact with the fused silica lens which is a part of the collimation system for the single photon counting system (please refer to Figure 1). Therefore the light observed on the photomultiplier tube would be a combination of scintillations originating from the polystyrene and the scintillation from the interaction of electrons with the lens. It is important to determine the relative contribution of each, and to think of this as an overall complex system of interaction, because in the case of Petri-dishes filled with cells and cellular media, the beta-particle flux on the lens would be expected to be reduced because of the interactions by beta particles in the cell medium, which will reduce the count rate resulting from the lens itself compared to the control measurements.

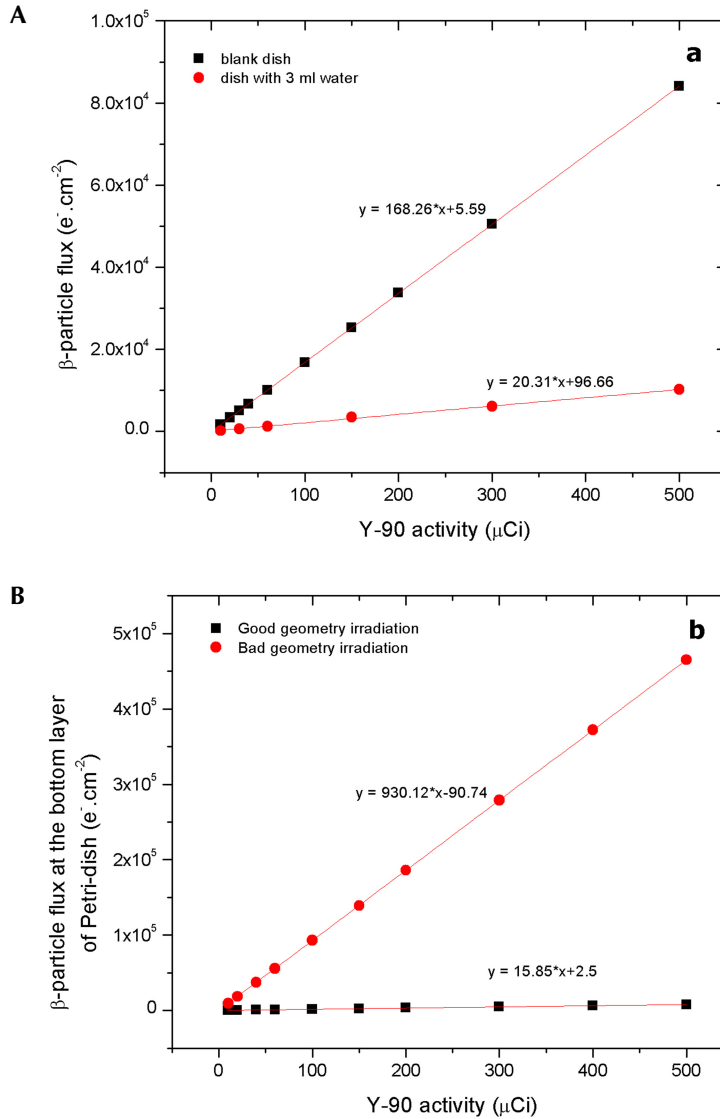


**FIGURE 2.** Light output against the <sup>90</sup>Y activity. (a) Light output when there is a 1.76 mm thick polystyrene substrate present between the source and the lens in the poor geometry condition. The solid line indicates a logarithmic fit according to equation 1. (b) Light output for both good and poor geometry conditions. In this case the observed light is shown for the two different thickness of polystyrene substrate. The black squares indicate the observed light without any polystyrene substrate.

Figure 2a shows the observed luminescence from the empty 35 mm diameter dishes for a range of <sup>90</sup>Y activities. Here the count rate was observed to be varying according to the following relationship

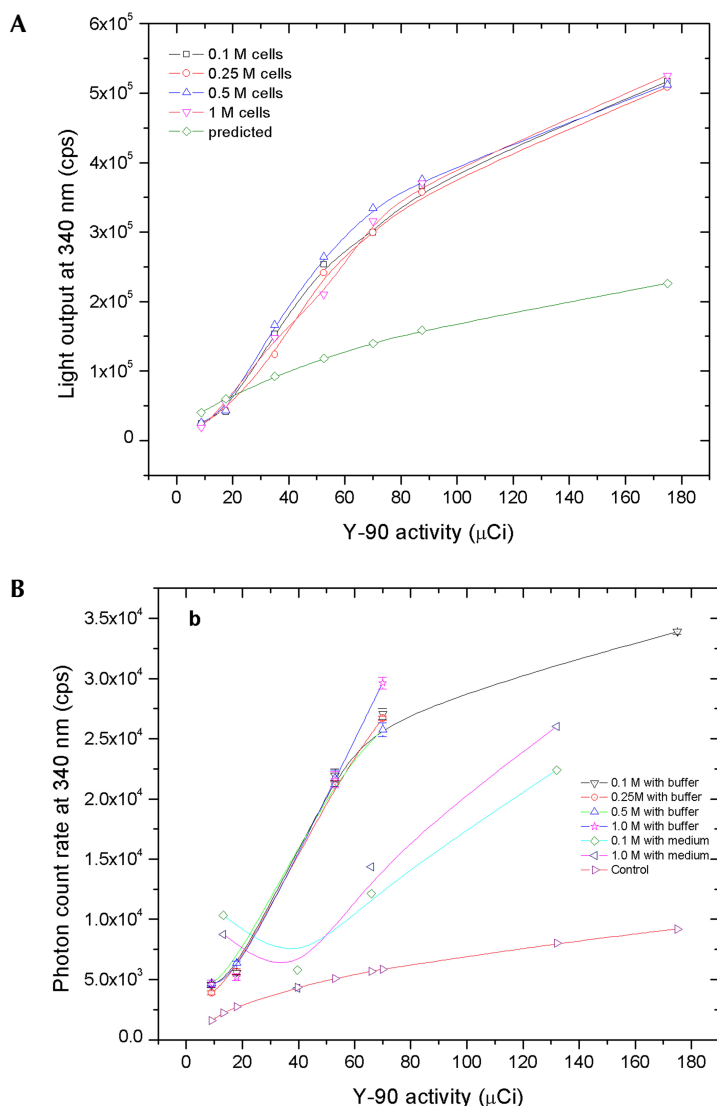
$$C.R = b \times \ln(A_o + c) \quad (1)$$





**FIGURE 3.** Monte-Carlo estimated  $\beta$ -particle flux for different activities of  $^{90}Y$ . (a)  $\beta$ -particle flux at the lens for a blank dish and a dish containing 3 ml water in it. (b) the  $\beta$ -particle flux at the dish surface in the good and poor geometry conditions

where  $a$ ,  $b$  and  $c$  ( $> 0$ ) are the fitting parameters  $C.R$  is the count rate and  $A_o$  is the activity. The fact that the lens in the collimating system was made of fused silica implied that there could be a significant proportion of scintillations coming from the lens itself. Fused silica has been studied for the emission of light using Sr-Y sources. It emits light at wide range of frequencies with varying intensities (Viehmann *et al.* 1975). In order to identify the relative contributions to luminescence from the lens we irradiat-



**FIGURE 4.** Light output from HPV-G cells, plated in different densities on polystyrene dishes, irradiated in the poor geometry condition for various  $^{90}\text{Y}$  activities. (a) Only cells (b) cells with clear buffer solution and coloured growth medium. The fitted trends in Figure 4 a and b are spline fits not indicating an overall pattern. M in the legend represents a Million.

ed it both in the presence and absence of the cellular substrate (polystyrene). Figure 2b shows the scintillations observed for both cases. For the poor geometry conditions there was a 4.35% (on average) increase in the observed count rate because of the presence of the cell substrate in between the lens and the source. This indicates that under the poor geometry conditions, the photon count rate measured by the PMT is

dominated by the scintillations occurring in the lens, most possibly because of scattered radiation (both beta particles and photons) reaching the lens.

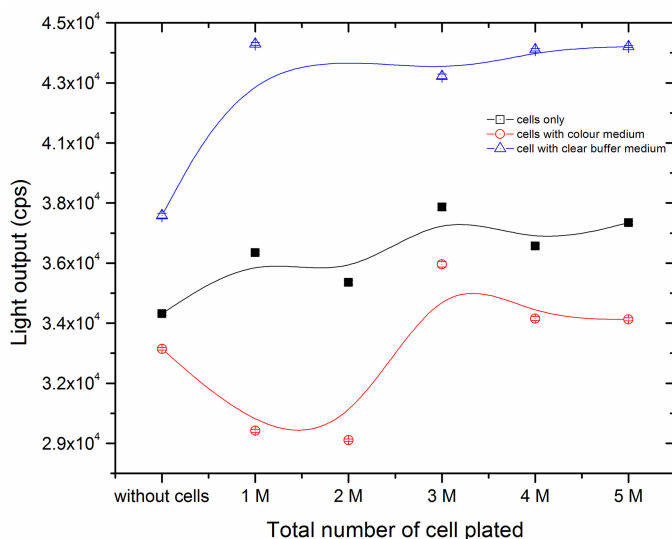
The high energy of the beta particles and the known scintillation of the lens glass under irradiation, led us to believe that for the poor geometry, the flux of  $\beta$ -particles on the lens would primarily determine the light output observed in the control experiment. In order to estimate this, the  $\beta$ -particle flux was calculated using the Geant4 Monte-Carlo toolkit. Figures 3a and 3b show the results of the simulations. The results in Figure 3a shows a predicted reduction in  $\beta$ -particle flux in the lens when the dishes are filled with cells and cellular media, and an estimate of the number of  $\beta$ -particles per unit area incident on the cells can be obtained using Figure 3b. For the sake of simplicity, we used water as a substitute for cell medium for the simulations. The height of cell medium was kept to approximately 3 mm above the cellular layer. The reduction in light output when water is added to the dish in the simulation results is attributed to absorption and scattering of the beta-particles in the water, reducing the number of beta particles incident on the lens.

The results of the simulation and the fact that the observed light output from the polystyrene substrate and the lens are almost indistinguishable led us to change the irradiation configuration, by using an aluminum collimator, with an aperture of 1 mm radius, in order to diminish the contribution to the light signal from scattered radiation.

As was previously shown, in Figure 2b, in the good geometry condition there was, on the average, a 37.6% and 41.2% increase in the counts in the PMT for 1.76 mm and 0.878 mm thick cell substrates respectively, compared to the case when the lens was irradiated directly with the  $\beta$ -particles from the source. This increase can be attributed to the light being emitted from the polystyrene substrate as a consequence of irradiation by  $\beta$  particles.

Figure 4 shows the results for  $^{90}\text{Y}$  irradiated HPV-G cells that were plated in different densities on the Petri-dishes in the good geometry condition. As mentioned earlier, these cells were killed using a 70% ethanol solution prior to the irradiation. Figure 4a shows the luminescence observed when the cells were plated in the dishes without any medium. Here the ethanol solution that was used to kill the cells was removed completely. Before irradiation, the cells were kept in a fume hood for a short period of time to allow any residual ethanol to evaporate.

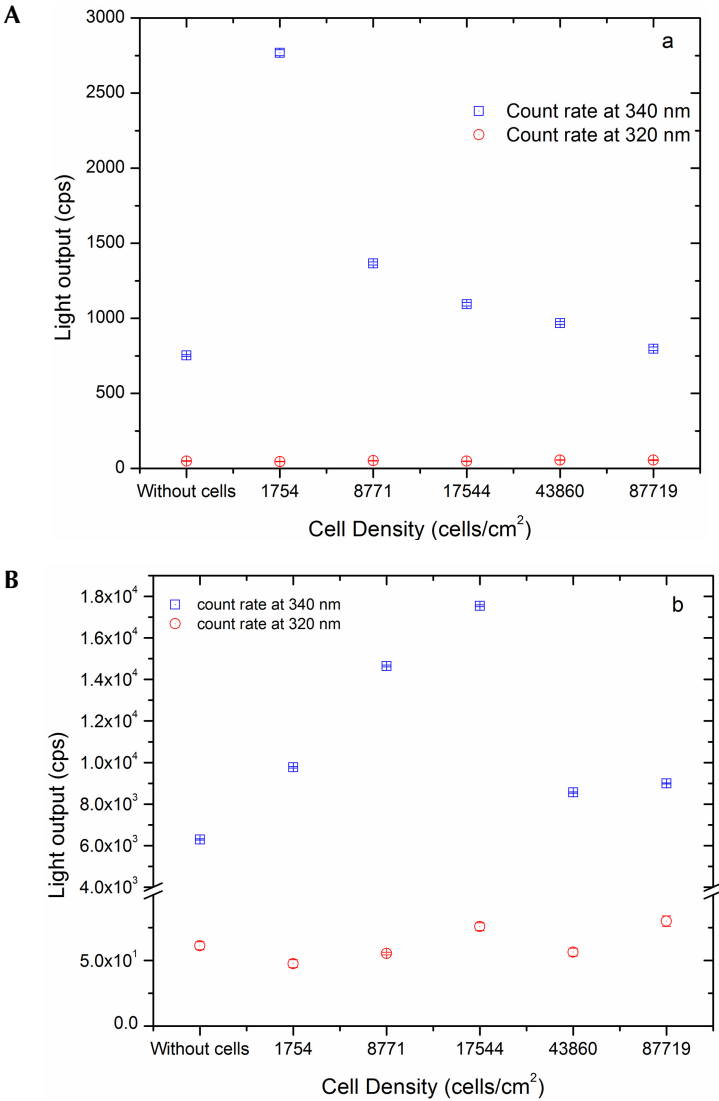
At the lowest levels of source activities, up to 40  $\mu\text{Ci}$ , it was observed that there was no evidence of significantly more light being emitted by the cells as compared to the control. In fact, for activity levels up to 20  $\mu\text{Ci}$  the light output observed for the control is approximately 34% higher than the light output observed from Petri-dishes with HPV-G cells. This suggests to us that at low activities, the absorption and scattering of light



**FIGURE 5.** Luminescence yield from cell plated in different densities on 35 mm diameter Petri-dishes.  $^{90}\text{Y}$  activity used was 398  $\mu\text{Ci}$ . Fit is merely a spline fit indicating the overall trend. M represents a Million.

in the cells dominates any increased emission by the cells. For higher activities, the light output increases with activity with all densities of cell plating showing significantly more UV emitted than from the Petri-dish alone. This indicates, to us, that the cells plus media are emitting UV as a consequence of their irradiation by beta-particles. The UV emission for a source activity of 180  $\mu\text{Ci}$  was observed to be 250% higher for cells plus dish than dish alone on average. The Monte Carlo calculated flux of  $\beta$ -particles to which the cells would be exposed varied between  $9.31 \times 10^3$  to  $1.67 \times 10^5$   $\beta \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  for the source activities mentioned in Figure 4 a&b.

We also irradiated cells in the presence of their growth media. For this purpose we looked at two solutions: the RPMI 1640 growth medium which is a coloured solution, and a simple buffered transparent liquid medium. We added 3 ml of solution to the dishes, which created a 3.12 mm thick layer of the solution on top of the cells. Figure 4b shows the variation of the measured light yield for a variety of cell densities in the Petri-dishes ranging from 0.1 M to 1.0 M cells. It can be observed that the light output in the case of the cells containing the coloured growth medium is much less than the light emitted from cells with the clearing medium. We suggest this clearly indicates absorption in and scattering by the coloured medium. There was no serum or nutrients present in the clear medium. This could also be a reason for the difference of signals emitted by both media, as the presence of serum or nutrients in the medium would change the photon absorption characteristics of the medium. It



**FIGURE 6.** UV output from cell irradiation with fixed activity using a collimated beam of  $^{90}\text{Y}$   $\beta$ -particles. (a) cell only and (b) cells with medium. Light output was determined at 340 nm and 320 nm. The background count rate was 45 cps. The 320 nm signal is not significantly different from the background rate.

can also be noted that in both Figures, 4a and 4b, the variation of light output from different cell densities is not significant enough in order to draw a useful conclusion about light output from the cells as opposed to the medium.

Since we were interested in determining the scintillation occurring in both, the cells and the growth media, our control was Petri-dishes alone. Using Figure 2a and Figure 3, we calculated the expected light output

from the cell substrate material and the lens. This is the curve plotted as “control” in each figure.

In order to determine the behavior of low LET radiation induced observed light output for varying cellular densities, we performed experiments at a fixed activity and irradiated cells plated in the range of  $1 \times 10^6$  to  $5 \times 10^6$  cells in the 35 mm diameter dishes. The flux of  $\beta$ -particles on the cells was estimated to be  $3.70 \times 10^5 \text{ } \beta \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  for this experiment. Although the light output observed from the cell containing dishes is significantly higher than from the control dishes, and there are light output differences that depend on the colour of the buffer solution, shown in Figure 5, there is no apparent relationship between light output and cell density under these particular conditions. For the cells with the coloured growth medium, the light output observed at cell densities of  $1 \times 10^6$  and  $2 \times 10^6$  was lower than that for the control. We attribute this to absorption and scattering of light by the coloured buffer, including light emitted from the polystyrene dish. The addition of coloured medium consistently reduced the measured light output, while the addition of clear solution increased light output compared with cells alone, which we attribute to a greater volume of irradiated material.

We considered the fact that scattered radiation may be the reason that no relationship was observed with cell density. We therefore designed a further experiment so that a collimated beam of  $\beta$  particles would irradiate the cells in an area that can be treated as a point source for the photon collimating lens. The cells were seeded in large dishes (100 mm diameter) in the range from  $0.1 \times 10^6$  to  $5 \times 10^6$  total cells in order to obtain a much wider range of cellular densities across the whole substrate. Figure 6 shows the observed light output plotted against cell density. The cell density represented on the x-axis is only a rough estimation based upon the total number of cells and the area of the dish. The  $\beta$ -particle flux at the Petri-dish in the presence of aluminum shield was determined to be  $7.06 \times 10^3 \text{ particles/cm}^2 \text{ s}^{-1}$  using Geant4 for an activity of 445  $\mu\text{Ci}$ .

It can be observed in Figure 6a that as the cell density increases in the dish (with no medium added) the observed count rate for 340 nm wavelength photons decreases in a crudely exponential manner. We attribute this to greater absorption and scattering of the photons by the cells, which outweighs the increased emission because of a larger cell density. The luminescence was also studied for wavelengths of 320 nm, however, it was not found to be significantly different from the background count rate. Figure 6b shows the relationship between light output and cell density when medium is added to the Petri-dish. In this case, the light output did not decrease in the same manner as for cells alone. We suggest that this may be due to the complex interplay between  $\beta$  interaction, UV emission, UV scattering and UV absorption properties of the cell/medium mix as the cell density within medium changes.

## DISCUSSION

The experiment presented in Figure 2 was originally designed to measure the light output from the interaction of energetic  $\beta$ -particles with the polystyrene cellular substrate of the Petri-dish. Light emission from polymers as a consequence of  $\beta$  particle and other ionizing radiations is not a new phenomenon and has been studied, generally for the purposes of radiation detection (Phillips and Schug 1969, Chen *et al.* 2011). It is, of course, the physical basis of many scintillation radiation detector systems. Generally, the scintillation response of most plastic scintillators to the total deposited electron energy is supposed to behave linearly (O'Rielly *et al.* 1996). In our data, presented in Figure 2a and Figure 2b, the light output appears to be deviating from linearity above 100  $\mu\text{Ci}$  in one case, and following a general linear behavior in the other. One reason may be a limitation by having the source in the form of a high specific activity: there could have been source thickness effects. Additionally, since the source was in the form of a liquid, the total volume to represent the same activity might have been different between the two situations, as fresh sources were made for each experiment. A larger volume of the source to represent the same amount of activity would mean that the source would be spread over a larger area on the Petri-dish. A lesser spread of the source on the Petri-dish would better represent a point source while larger spreads would have created an area source. This could lead to discrepancies between experiments. In later experiments, we tried to keep the specific activity constant so that discrepancies due to the volume of the source could be minimized.

The experiments illustrated in Figures 4 and 5 indicate that the addition of cells and/or cell medium to the Petri-dish results in a statistically significant change in the observed count rate for UVA photons at 340 nm. A relationship between UVA output and cell density was not clear. In this experiment the dishes were exposed to a fluence of  $0.4 \times 10^6$   $\beta$ -particles/ $\text{cm}^2$  and the cell densities varied from approximately  $0.1 \times 10^6$  cells/ $\text{cm}^2$  to  $0.5 \times 10^6$  cells/ $\text{cm}^2$ . A two way analysis of variance performed for the data presented in Figure 5 investigating the difference between cells alone and cells with clear cell medium added found a statistically significant difference between the light output of cells and cells plus medium ( $p < 0.001$ ). Adding clear cell medium increased the measurable UVA signal. A two way analysis of variance performed for the data presented in Figure 5 investigating the difference between cells alone and cells with coloured cell medium added found a statistically significant difference between the light output of cells and cells plus coloured medium ( $p < 0.001$ ). Adding coloured cell medium decreased the measurable UVA signal. In both cases, there was a suggestive, although not significant ( $p=0.1$ ), effect of cell density on the signal. These results make a certain

amount of sense. Adding coloured medium would be expected to increase absorption and scattering and reduce the signal at the detector, while the addition of clear medium adds more material in which to generate UV emission, with less of an effect on absorption and scattering.

After rearrangement of the experimental setup, by introducing an aluminum collimator, increasing the range of cell densities and keeping the fluence to  $7000 \beta\text{-particles}/\text{cm}^2$ , we observed an effect of cell density when cells alone were studied. Increasing the number of cells in the dish (while not adding cell medium) reduces the measured light output. The observed count rate reduces by a factor of 2 as the cell density changes from  $0.1 \times 10^6$  cells to  $0.25 \times 10^6$  cells per dish. Overall, the effect of increased cell density appears to be a crudely exponential reduction in UVA measured at the PMT. There are only 5 data points, so it is not possible to determine precisely the exact mathematical shape of the relationship. We suggest that the increase in the number of cells increases the absorption and scattering of photons. We observed the light output at 340 nm but not at 320 nm. The count rate observed at a wavelength of 320 nm was equal to the background rate. We therefore suggest that the emission that arises as a consequence of irradiation is predominantly for wavelengths equal to or higher than 340 nm. When medium was added to the dishes, the relationship between cell density and light output was less clear. Further measurements and refining of the experimental process are clearly required.

We focused on measuring the photon emission at a wavelength of 340 nm, that is, in the UVA range, from HPV-G cells irradiated with low LET  $\beta$ -particles. In our previous studies, we had demonstrated emission of UV photons as a consequence of irradiation by high LET radiation from materials used as cell substrates and materials derived from living cells. We wanted to determine whether cells and cell media also emitted UV as a consequence of irradiation, and as radiation induced bystander effects studies employ both high and low LET radiation, we studied low LET in this instance.

We believe the preliminary data presented here demonstrate emission of UVA from cells, cell media and substrate materials commonly used in radiation biology as a consequence of irradiation with  $\beta$  particles. Of course, the complex molecular structure of biological media means that in addition to emission of UVA there is also absorption and scattering of UVA by cells, media and substrate materials. The resultant external measurement of UV signal at the PMT is therefore a result of several complex interaction processes. It would be interesting to expand these measurements for heavier charged particles and perhaps indirectly ionizing radiation. Type of cells could also influence the absorption of secondary radiation in the cells or cell media and would consequently affect the emission characteristics as well.



However, the fact that we observed measurable emission of UVA photons as a consequence of  $\beta$  irradiation has potentially important consequences for the understanding of radiation bystander effects: especially the understanding of effects that have previously been believed to be chemically mediated. We measured UVA which, once absorbed in the cells, can lead to a variety of known biological end points. Several studies have shown cell mutations, DNA damage, chromosomal aberrations, and alteration in the cell signaling pathways as a consequence of UV irradiation especially in the long wavelength range (Emri *et al.* 2000, Kappes *et al.* 2006, McMillan *et al.* 2008). UV-A produces damage by creating reactive oxygen species and has also been shown to produce the bystander effect (Banerjee *et al.* 2005, Whiteside and McMillan 2009, Nishiura *et al.* 2012),. Many bystander effect studies still emphasize the presence of a biological or chemical signal that diffuses through the medium and causes the observed effects. A strong reason for this belief is the observation of effects in medium transfer bystander experiments. However, we suggest that one of the difficulties in recent interpretation and understanding of the “bystander effect” is that it is, in fact, not a single effect but several effects which result in similar endpoints. The medium transfer experiments do indeed suggest a chemically mediated signal. However, the preliminary data we present here, suggest, we believe, that there is a “physical” bystander effect. UVA that is emitted along the track of ionizing radiation can potentially interact with cells that are not targeted by the original radiation and create effects at a distance from the targeted cells. This study could also have an impact on the in-vitro studies, as the primary charged particle track would still be accompanied by secondary radiation in the form UV photons.

## CONCLUSIONS

We have presented data for emission of UVA from biological media when it is subjected to low LET radiation. In this case we used  $\beta$ -particles from a source of  $^{90}\text{Y}$ . Initial experiments showed that the presence of cells or their medium in a Petri-dish significantly increases the observed count rate of photons at a wavelength of 340 nm as compared to an empty control Petri-dish. Coloured growth medium added to the cells reduced the count rate, while a transparent buffer in equal volume increased the count rate, as compared to cells alone. This provided evidence that emission, scattering and absorption of light were factors in the experiment. Under collimated irradiation conditions, it was observed that increasing cell density resulted in a decrease in the observed light output. UVA would appear to be emitted by cells, cell medium and cell substrates when irradiated with low LET radiation. However, the overall process is complex. Cells, medium and cell substrates appear to emit light at UVA frequencies under  $\beta$ -particles irradiation but also absorbed and scattered

light. We suggest that the emission of UVA as a consequence of ionizations may explain some radiation bystander effects that have previously been attributed to a chemical signal. Further work is clearly necessary to investigate this phenomenon further.

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